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Research Article

Life history and behavioural observations during the rearing of *Dira clytus clytus* (Linnaeus, 1764) (Insecta, Lepidoptera, Nymphalidae), with notes on implications for climate change adaptation

Silvia Mecenero[1], Stephen P. Kirkman[2]

1 *Lepidopterists' Society of Africa, Somerset West, South Africa*
2 *Institute for Coastal and Marine Research, Nelson Mandela University, Gqeberha, South Africa*
Corresponding author: Silvia Mecenero (silviakirkman@gmail.com)

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Abstract

Lepidoptera are known to alter their behaviour and phenology in response to climatic conditions. Understanding these responses is important for the conservation of Lepidoptera species, especially those that are not widely distributed. Individuals of *Dira clytus clytus*, a South African endemic butterfly with a distribution in the southern parts of the country, were reared in captivity from the egg phase to the adult phase, to observe behaviour and phenology in the various life stages. Life history stages concurred with previous observations made on this subspecies, but additional photographs are presented for the various phases. There are clearly two pupal, and thus adult emergence, phases in spring and early summer. Four batches of eggs resulted from these adults, and the larvae from these eggs were also reared although none of them survived to the pupation phase. The timing of the two pupal phases was compared to climatic variables (temperature and rainfall) and it was found that pupation followed soon after cold temperature. This is indicative that *D. c. clytus* can change its phenology and voltinism in response to climatic conditions, which may allow for plasticity in responding to climate change. However, more research is required to understand how climate change would impact this endemic species.

Key words: Climate, diapause, phenology, satyrine, voltinism

Introduction

Understanding the behaviour and phenology of Lepidoptera life history stages in relation to climatic conditions gives insights into their adaptability and vulnerability to changing environmental conditions (Stefanescu et al. 2003; Hill et al. 2021; Zografou et al. 2021; Buckley 2022). This can subsequently allow for appropriate conservation interventions to improve the responses of Lepidoptera to climate change impacts, and is especially relevant to species that are not widely distributed. One such species is the satyrnine *Dira clytus*, which is one of four species within the genus *Dira* that are all endemic to South Africa (Mecenero et al. 2013)

The subspecies *D. c. clytus* is mainly found in the southern parts of the Western Cape province, whereas subspecies *D. c. eurina* is found in the Eastern Cape province (Mecenero et al. 2013). *Dira clytus clytus* has a flight period that ranges from February to May, with a peak in March (Trimen and Bowker 1887; Van Son 1955; Williams 2020). The adults fly above grassy slopes and lower ground and, within urban areas, above plots of long Kikuyu grass (*Pennisetum clandestinum*) (Van Son 1955; Pringle et al. 1994), being active in the morning until about midday (Quickelberge 1978) and with males seemingly more active and numerous than females (Pringle et al. 1994). Trimen and Bowker (1887) described the morphological differences between male and female adults of this species, as well the morphology of the pupa, but provided no information on the larva or its life history or food plant. The most comprehensive description of the life history of this species was made by Clark (1943), which included information on the eggs, all five larval instars and their food plant, the pupae and adults.

This study presents life history and behavioural observations made during multiple captive rearing events of *D. c. clytus* larvae over a year, in contribution towards the Caterpillar Rearing Group project (Lepidopterists' Society of Africa), specimen code SM19_01. The study complements previous observations of this species made by Trimen and Bowker (1887), Clark (1943), Van Son (1955) and Quickelberge (1978), with the addition of photographic evidence. The implications of the observations made in the phenology of the life stages for climate change adaptation are discussed.

Material and methods

On 8 April 2019, eight *D. c. clytus* adults (Fig. 1a) were captured with a net in Geelsloot Park (34°04.55'S, 18°50.83'E), Somerset West, in the Helderberg area of Cape Town, South Africa. They commonly occur here as a small colony every year (mainly during April), flying slowly just above a small grassy plot where they are usually observed in the early morning (not seen later in the day). The captured adults were kept in a small, netted enclosure for one day in order to collect eggs, along with cuttings of various grasses growing in the grassy plot. Numerous eggs were collected later during the same day (Batch 0 eggs). The eggs were found scattered on the floor of the enclosure – none were found on leaves of grass or any other surface except the enclosure floor. The adults were released back into the grassy plot.

Once the eggs hatched, larvae were presented with the four different grass species found growing at the grassy plot: *Pennisetum clandestinum*, *Bromus catharticus*, *Paspalum dilatatum* and *Digitaria sanguinalis* (all four exotic to South Africa). Larvae were reared through to adults indoors, by keeping them in plastic containers with ventilation holes on the lid. Larvae were fed fresh cuttings of their preferred grass daily. Pupae were transferred into netted enclosures. Once the adults emerged, they were kept in the netted enclosures until eggs were laid and then they were released. Four additional batches of eggs were collected (Batches 1–4) and larvae that hatched from these eggs were reared similarly to the larvae from Batch 0 eggs. All life history stages were photographed.

The timing of pupation was compared with daily average temperature (°C) and rainfall (mm) data for the Helderberg area obtained from the South African Weather Service.

Results

Morphology

The eggs that were laid by adult butterflies (Fig. 1a) were dome-shaped with an indentation on one side and pale yellow (Fig. 1b). Five larval instars (Fig. 1c–l) and the pupal phase (Fig. 1o, p) were observed and photographed.

Phenology and behaviour

All the Batch 0 eggs that were laid on 8 April 2019 hatched on 21 April 2019, with the egg phase lasting 13 days. About 185 larvae hatched out from Batch 0 eggs. All the larvae selected *P. clandestinum* as their food plant and started feeding on it. None of the larvae selected the other three grasses presented to them as a potential food plant.

There were individual variations in the timing of changing instars and it was not possible to keep track of all larvae, however, the following changes in instars could be approximated from the observations: the first larval instar lasted approximately 9 days and prepared for moulting around 30 April; the second instar lasted approximately 15 days and prepared for moulting around 15 May; the third instar lasted approximately 17 days with the change to the fourth instar occurring around 2 June; the fourth instar lasted approximately 32 days with the fifth instar starting around 4 July. On 4 July 2019, the first observation was made of the larvae huddled very close together in groups (Fig. 1m) and this behaviour occurred frequently throughout the cold season.

Prior to pupation, the larvae curled up and remained in a motionless state for 1–2 days before pupating (Fig. 1n). Pupae were found on the bottom of the container, not attached to any substrate. The first three pupae were observed on 10 September 2019, 143 days after the larvae hatched and approximately 69 days after the fifth instar formed. Two days prior to these pupae forming, there was a heat wave (> 30 °C). The first two adults emerged on 30 September 2019. In total, there were 59 pupae and 50 adult emergences from batch 0 eggs, resulting in a 27% survival of larvae to adults in captive conditions. In Fig. 2, the dates that larvae from Batch 0 eggs pupated and that adults emerged are shown. There are clearly two pupal and adult emergence phases:

1. In the first phase, 12 pupae were observed from 10 September to 30 September 2019 and adult emergence was observed from 30 September to 21 October 2019. The pupal phase was 20 to 21 days. All 12 pupae eclosed (100% eclosion).
2. In the second phase, 46 pupae were observed from 2 November to 18 November 2019 with one final pupa forming on 27 November 2019. Adult emergence was from 23 November to 14 December 2019. The pupal phase ranged from 17 to 21 days, up to three days shorter than the first phase. Thirty-eight of the pupae eclosed (83% eclosion). Compared to the first phase, this second phase had approximately four times more pupae and approximately three times more adult emergences.

From the moment that larvae hatched, they consistently ate a lot of the food plant until about mid-September, at which time they were in the fifth instar (some larvae had already pupated, see above). These remaining fifth instar larvae then

Figure 1. Photographs of the life stages of *Dira clytus clytus* taken during its rearing **a** adult **b** eggs **c, d** newly hatched larva (dorsal and lateral views) **e** first instar larva (three days old) **f** first instar larva preparing to moult (nine days old) **g, h** second instar larva (dorsal and lateral views) **i** third instar larva **j** fourth instar larva **k, l** fifth instar larva (dorsal and lateral views) **m** fifth instar larvae huddling together in a big group **n** pre-pupal form **o, p** pupa (lateral and ventral views).

apparently ceased feeding and remained in a sluggish, immobile state. Some pupated by 30 September (first phase of pupation described above) but the majority remained in this immobile state until 25 October (over a month), at which point they suddenly began to move actively around the container. One day prior to them becoming active, it rained heavily (>20 mm) and it was very cold (minimum temperature 13 °C). About a week later the second pupal phase described above started.

By 30 October, the remaining larvae became immobile and stopped eating again, until 19–20 November (approximately three weeks). During this immobile period, a greater number of individuals pupated (the second phase of pupation described above). The remaining larvae then became active again on 20 November, the day after a cold day (max 17 °C), and moved around actively, though they ate very little of the grass (a few tiny nibbles). A few seemed to be getting ready to pupate but only one more pupa formed on 27 November 2019. The remaining larvae died.

The two pupal phases described above started 5–7 days after very cold weather lower than 15 °C average temperature (Fig. 2). The second pupal phase, where more pupae developed than the first pupal phase, also followed about 9 days after heavy rainfall (Fig. 2).

Adults that emerged from the rearing of larvae from Batch 0 eggs, laid eggs at four different times (Batches 1–4) (Table 1). All the larvae that hatched from Batches 1–4 eggs died prior to pupation.

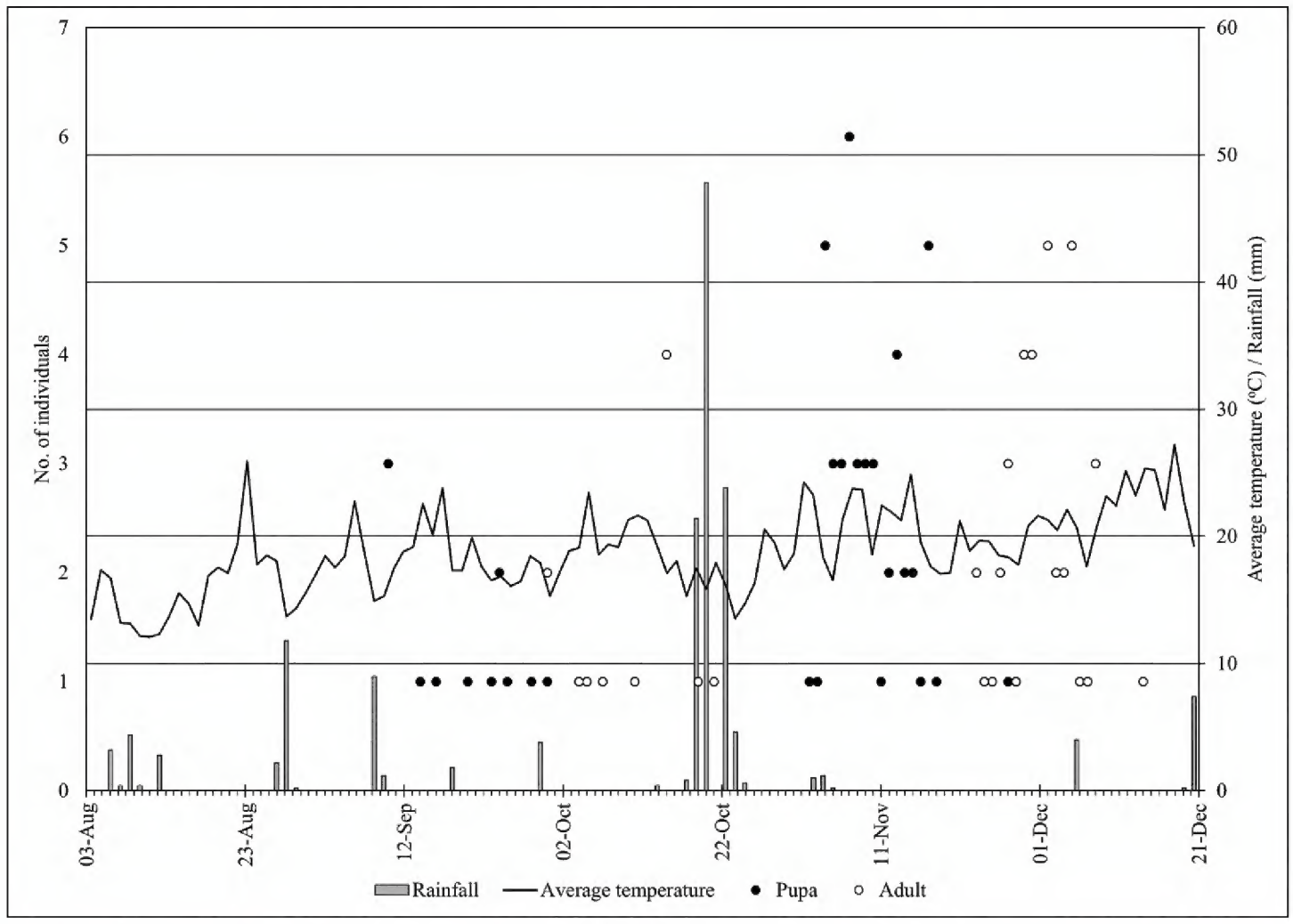

Figure 2. Number of pupae (black dot) and adults (white dot) from batch 0 eggs compared to average temperature and rainfall.

Table 1. Egg laying and hatching dates, and egg phase length, of eggs laid by Batch 0 adults.

Egg batch	Laying date	Hatching date	Egg phase length (days)	Number of larvae hatched
Batch 1	9 October 2019	20 October 2019	11	28
Batch 2	17 October 2019	29–31 October 2019	12–14	110
Batch 3	30 November 2019	12 December 2019	12	- (all died by mid-January)
Batch 4	1 December 2019	20–21 December 2019	19–20	- (not counted)

Discussion

Dira clytus clytus was successfully reared in captivity. The egg, larval and pupal stages that were observed and photographed correlated with the descriptions and diagrams of Clark (1943). Adult females laid eggs by scattering them, not by laying them on a substrate, supporting previous observations (Clark 1943; Quickelberge 1978).

The length of the egg phase of the first batch of eggs (Batch 0) fell within the range reported by Clark (1943). The egg phase from subsequent batches (Batches 1–4) ranged from 11–20 days increasing Clark's (1943) range by four days. Clark (1943) indicated that the length of the egg phase varies with the weather, but no clear pattern could be seen, e.g. eggs laid on 30 November hatched 12 days later, but those laid a day later on 1 December hatched 19–20 days later (Table 1).

The food plant was *P. clandestinum*, which has been mentioned as a food plant for this species in suburban settings (Pringle et al. 1994). This grass is also a food plant for *D. jansei* and *D. swanepoeli* (Williams 2020). None of the other three grasses rejected by the larvae are listed as food plants for *Dira* species (Clark 1943; Williams 2020).

The instar and pupal phases followed similar phase lengths as described by Clark (1943). In the wild, Clark (1943) found fully grown larvae in crevices and under stones, lying inertly. Similarly in this study, fully grown larvae were found to be sluggish and immobile at the bottom of the containers for long periods

of time, eating very little if at all. Pupae formed on the bottom of the containers and none were attached to a substrate, which supports previous observations made (Trimen and Bowker 1887; Clark 1943).

The huddling behaviour exhibited by the captive larvae during the colder time periods is likely for thermoregulatory purposes to conserve heat (Klok and Chown 1999). It is unknown whether the larvae huddle in the wild, where they are able to hide in crevices and in between grass blades (Clark 1943), thereby potentially reducing heat loss, unlike in the captive conditions where crevices were lacking.

The larvae of this species are known to go into diapause, pupating a few weeks prior to when adults emerge from February to May with a peak in March (Trimen and Bowker 1887; Van Son 1955; Williams 2020). Diapause occurs in the fifth instar, as observed by this instar phase being the longest of all the instar phases (64 days and longer) and the larvae being mostly sluggish, immobile and not feeding much during this phase. Also, for the 50 pupae that eclosed, the pupal phase ranged from 17–25 days, indicating that this butterfly does not diapause during its pupal form.

Accordingly, pupation occurs a few weeks prior to the appearance of adults. It is unclear what triggers pupation, but it is likely due to a drop in temperatures towards late summer. According to Quickelberge (1978), adults of *Dira* species are stimulated to emerge when there is a drop in temperature ahead of the approaching winter months. The same author found that the conspecific *D. c. eurina* emerges 1.5 months later at sea level than at localities 80 km away at higher altitude and which therefore experience cooler temperatures earlier as winter approaches. The pupal phase in this study varied little in length, therefore it is likely that environmental cues stimulate larvae to pupate, rather than stimulating the adults to emerge. It is possible that very cold temperatures (< 15 °C) may stimulate larvae to pupate, despite the temperatures warming up as summer approached. This may explain the two pupal phases during spring and early summer that followed shortly after average temperatures of < 15 °C were experienced.

In the wild, *D. c. clytus* is single-brooded, with adults absent for a period of nine months (Clark 1943; Van Son 1955; Quickelberge 1978). However, observations have been made of adults flying out of season. For instance, two adults were seen flying in Knysna, South Africa, during late December (Quickelberge 1978) and a few adults during mid-June (Clark 1943). This study has shown that captive-reared *D. c. clytus* have the ability to produce at least two broods per year (in September and November in this study), with an interval between adult emergences. Unfortunately, the remaining larvae died prior to the end of summer, which would have been the normal timing of pupation and a thus third emergence period. Clark (1943) stated that under favourable conditions, some larvae will pupate instead of entering diapause. It is interesting to note that in captivity the adults emerged 4–7 months earlier than usual, while no adults were observed flying by the grassy plot during the same time period, which is about 30 m away from where they were reared. None of the larvae from the second brood resulted in pupae or adults, so it remains unknown if a third brood would be possible. Other satyrines in Sweden display variability in their phenology, such as reduced growth rates in the later larva instars and aestivation, due to seasonal cues (Wickman et al. 1990). There may be specific environmental conditions (e.g. temperature, humidity, day length) that are required for the successful completion of the life cycle, and this may be the reason why the second brood failed in captivity, and why this butterfly is single-brooded in the wild.

Insect growth and development is sensitive to temperature (Buckley 2022). Shifts in climate and thus temperatures can affect the phenology of Lepidoptera, such as early emergence from diapause and increases in the annual number of generations (increasing voltinism) (Stefanescu et al. 2003; Hill et al. 2021; Zografou et al. 2021; Buckley 2022). The observations in this study suggest that *D. clytus* may be able to respond to changing climatic and seasonal conditions by pupating, and thus emerging, earlier than usual and by increasing voltinism. Nevertheless, such plasticity in phenology may not always be beneficial to Lepidoptera, with populations potentially being more vulnerable to extreme and rapid changes in climate (Hill et al. 2021). Also, there may be mismatches with the availability of host plants, and the more host-specific a species is, the greater the risks of phenological shifts may be (Hill et al. 2021). Specialists are more vulnerable to changes in seasonality and climate change, with generalists being more adaptable to changing environmental conditions (Zografou et al. 2021; Löckinger et al. 2024). The latter tend to have more than one generation a year and hibernate at a more advanced stage of development (Börschig et al. 2013). *Dira clytus* feeds on a variety of grasses including *P. clandestinum* commonly found in suburban settings and may therefore be more flexible to changes in its phenology. It also exhibits diapause in the final larval instar phase, which may facilitate its earlier pupation and thus emergence. Further observations are required to determine the impact of changing seasonality on this endemic species.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

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Author contributions

Silvia Mecenero: Conceptualisation, Methodology, Writing - Original draft. Stephen Kirkman: Analysis, Writing - Review and Editing.

Author ORCIDs

Stephen P. Kirkman https://orcid.org/0000-0001-5428-7375

Data availability

All of the data that support the findings of this study are available in the main text.

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